Cyclization in concert

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The effect is dependent on the particular species; similar experiments with *E. coli* exhibited no such spatially dependent enhancement. The effect, termed 'quorum acting', differs from the well-known phenomenon of quorum sensing[7] in that the latter is defined by alterations in the cellular phenotype itself, whereas this new effect is characterized by alterations to the external environment induced by spatial localization of the cells.

Microfluidic devices enable the study of various phenomena in the presence of precisely controlled flow conditions, and Kastrup *et al.*[3] used this capability to demonstrate that clusters of *B. cereus* still initiate coagulation in the presence of flowing whole blood. Experiments also confirmed that specific coagulation factors must be present for the quorum acting process, while others are not essential, and therefore, a coagulation network in which quorum acting bypasses some of the initiation points can be mapped. Numerous *Bacillus* species activate coagulation *in vitro*; one interesting finding is that *B. anthracis* requires a specific metalloprotease in order to trigger coagulation. Mouse models injected with *B. anthracis* also exhibited a strong correlation between the bacterial clustering and coagulation, but differences between mouse and human infection and pathophysiology complicate the picture considerably.

The ability of microfluidic models to simulate coagulation-related phenomena is an exciting advance with many potential applications in basic research and in the development of new therapeutic approaches. Septic shock and related conditions have long been associated with coagulation, and yet a complete picture of the mechanisms activating and controlling this correlation has not emerged. With new insights into quorum acting phenomena and the ways in which bacteria communicate to influence not only their own behavior but also that of the local environment, a deeper understanding of infection-induced coagulation is possible. Many questions remain, however, as effects such as coagulation in the presence of *B. anthracis* seen *in vitro* and in animal models may not be consistent with recent human clinical experience[8]. Future work along the lines of Kastrup *et al.*[3] is likely to be very instructive in addressing key questions, such as the relationship between bacterial infections and cardiovascular disease, the evolution of cooperative effects within host responses and within bacterial populations, and approaches towards reducing the incidence of coagulation related to microbial contamination and biofilms in vascular access management.

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Marco W Fraaije & Andrea Mattevi

The berberine bridge enzyme catalyzes the crucial step in the biosynthesis of an important class of alkaloids through a reaction that cannot be carried out using conventional organic chemistry tools. Characterization of the enzyme demonstrates a concerted mechanism that couples two distinct chemical steps—oxidation and proton abstraction—affecting two separate groups of the substrate.

Berberine bridge enzyme (BBE, Enzyme Commission number 1.21.3.3) catalyzes a key reaction in the biosynthetic pathways toward a major and diverse class of plant alkaloids—the benzophenanthridines. These molecules have been widely studied because of their many potential applications that range from hypotension treatment to anticancer activity[1]. Consistently, there is a growing demand to understand and engineer cellular and biocatalytic systems that can efficiently synthesize these compounds[1]. BBE shows a highly refined selectivity and reactivity, as it specifically recognizes the *S* enantiomer of reticuline, which is converted into (*S*)-scoulerine by a regioselective cyclization reaction (**Fig. 1a**). This atypical oxidative cyclization generates a chemical link ("berberine bridge") between the substrate isoquinoline and benzyl rings, which results in the formation of the tetracyclic skeleton of berberine-related alkaloids. A crucial feature of the reaction is that such a selective modification cannot be achieved by synthetic organic chemistry. Therefore, BBE represents an invaluable biocatalytic tool for synthetic biology approaches aiming at the production of bioactive alkaloids[2]. The structural and biochemical analysis of the enzyme reported by Winkler *et al.*[3] in this issue of *Nature Chemical Biology* represents an important advance, providing conclusive evidence in support of an unprecedented mechanism of catalysis.

Though BBE activity was already known for several decades[4], the mechanism by which the berberine bridge is formed remained elusive[5]. The combination of mutagenesis of several active site residues, kinetic analyses and a crystal structure of the enzyme from *Eschscholzia californica* in complex with (*S*)-reticuline supports a mechanism in which a hydride anion is transferred from the *N*-methyl group of the substrate isoquinoline ring to the flavin group of the FAD cofactor. Furthermore, the authors demonstrate that the two-electron oxidation of the substrate is coupled to a regioselective proton abstraction from the substrate benzyl group by an essential glutamate residue (Glu417) (**Fig. 1b**). This concerted "proton and hydride" abstraction gives rise to a Friedel-Crafts–like alkylation, eventually yielding the ring-closed (*S*)-scoulerine. Key to this elegant solution for a seemingly complex reaction is the proper orientation of the substrate with respect to the two catalytic groups; the flavin cofactor, the *N*-methyl group of the substrate isoquinoline ring, the hydroxyl group of the substrate benzyl ring and the carboxylate of Glu417 are precisely aligned within the active site to promote catalysis[3]. This binding mode is at the heart of the catalytic mechanism uncovered by this study.

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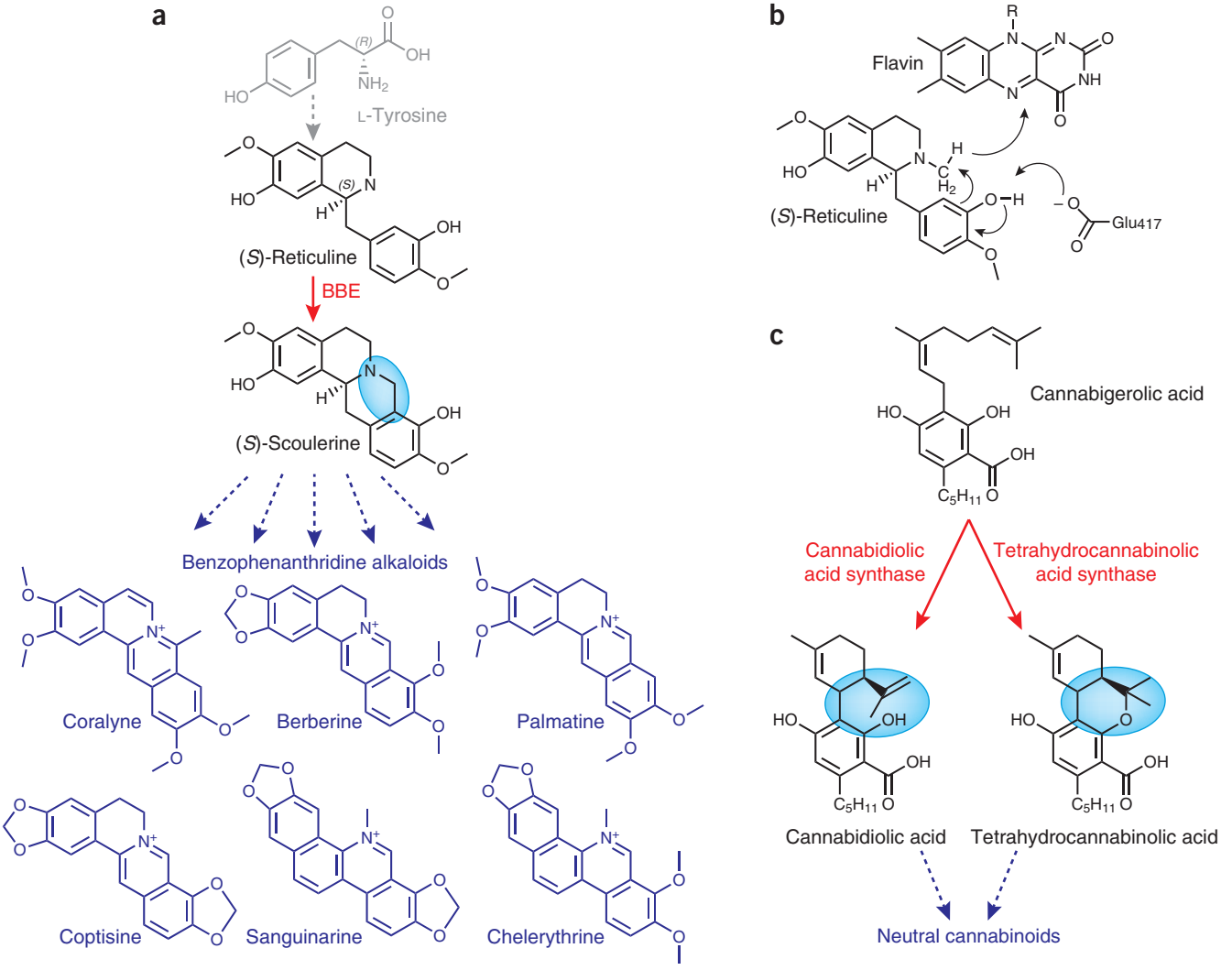

Figure 1 Biochemistry of BBE and homologous enzymes. (**a**) BBE catalyzes the conversion of (*S*)-reticuline to (*S*)-scoulerine, a cyclization reaction that involves the formation of the so-called berberine bridge that links the benzyl and isoquinoline rings of the substrate. Reticuline is synthesized from L-tyrosine, whereas the product of the BBE reaction represents the precursor of a wide and diverse group of alkaloids that have various biological activities. (**b**) Simplified scheme of the catalytic mechanism of BBE proposed by Winkler *et al.*[3]. (**c**) Plant genomes exhibit a number of BBE homologs whose biological activities remain mostly to be uncovered. Among the BBE homologs, there are enzymes that take part in the biosynthesis of cannabinoids. Remarkably, these synthases act on molecules that substantially differ from reticuline, highlighting the biocatalytic potential and diversity of the family of BBE-related enzymes.

The structural and mechanistic insights into BBE function are relevant for the analysis of related enzymes. In fact, plant genomes appear to be rich in BBE homologs. For instance, 12 homologs have been observed in the *Arabidopsis thaliana* genome[6]. This indicates that enzymes of the BBE family are likely to be involved in diverse and yet-unknown processes of plant metabolism, thus representing a reservoir of catalytic potential that remains to be explored. This notion is exemplified by the BBE homologs that have already been shown to take part in the biosynthesis of another class of plant alkaloids—the cannabinoids[1]. Δ-Tetrahydrocannabinolic acid synthase and cannabidiolic acid synthase from *Cannabis sativa* catalyze a BBE-like oxidative cyclization reaction while acting on compounds with a different scaffold with respect to that of reticuline (**Fig. 1c**)[7,8]. As both enzymes show high sequence homology to BBE (40% sequence identity), including conservation of the FAD-linking residues and the active site glutamate, these synthases are likely to use a similar concerted reaction that couples oxidation with proton abstraction.

A striking structural feature of BBE and the above-mentioned synthases is the mode in which the FAD cofactor is bound: it is covalently tethered to the polypeptide chain via two residues. Such an 8α-histidyl-6-*S*-cysteinyl-FAD cofactor has only been observed in a small number of recently reported oxidases[9]. Except for this characteristic mode of cofactor binding, members of this subclass of bicovalent flavoproteins also share another feature: they act on relatively complex molecules, often secondary metabolites. This may also relate to the rationale behind the double covalent FAD linkage. To create an active site that accommodates a bulky substrate molecule, while assuring appropriate positioning of residues and the flavin cofactor, a tight anchoring of the cofactor may be beneficial. Future studies on the recently discovered bicovalent flavoproteins will clarify the exact functional role of the double covalent anchoring of the cofactor.

Structural and mechanistic knowledge enables dedicated enzyme redesign by which new synthetic routes can be developed. In fact, one of the BBE mutants reported by Winkler

et al.[3] already shows formation of a new ring closure product, (*S*)-coreximine. Adapting BBE and related proteins toward other substrate types (by directed evolution for example) will aid synthetic biology approaches aiming at generation of new pharmaceuticals (see for example ref. 10). In fact, nature already illustrates such flexibility in selectivity, as BBE homologs of the cannabinoid metabolism act on compounds that are chemically and biologically unrelated to reticuline and similar alkaloids (**Fig. 1**). The results presented by Winkler *et al.*[3] and the recent successful attempts of metabolic pathway engineering[1] indicate that the biocatalytic potential of BBE and similar enzymes is still largely unexplored and will represent a major topic for future research.

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Seeing cellular sialidase transform sugars

Minoru Fukuda & Xingfeng Bao

Cell-surface carbohydrates are synthesized in a step-wise fashion, yielding products with unique capping structures. A recent study has shown that carbohydrates at the cell surface can be further remodeled by an endogenous glycosidase to alter the carbohydrate structure, thus generating a new function.

Carbohydrates serve important roles in eukaryotic cells, and in particular they function as intercellular regulators when displayed on the cell surface as protein or lipid conjugates. These often complex sugar structures are typically synthesized in the endoplasmic reticulum and Golgi apparatus by the step-wise addition of different carbohydrate residues onto the appropriate biological scaffold by glycosyltransferases. This occurs in the creation of mucin-like O-glycans, which are initiated by the addition of *N*-acetylgalactosamine to serine or threonine residues[1]. Unlike DNA and protein synthesis, however, carbohydrate synthesis does not always proceed in a forward direction; instead, individual or several sugar residues can be removed, or 'trimmed', from a growing chain while in the endoplasmic reticulum and Golgi. This processing is well known, for example in the case of converting mannose-rich N-glycans to precursor N-glycans in the Golgi by mannosidases, a required step in converting the carbohydrates present in yeast to the more complex N-glycans in animal and plant cells[2]. The significance of appropriate carbohydrate modification is illustrated by neonatal lethality in mice with inactivated α-mannosidases[3]. A new study now demonstrates a surprising link between these systems in describing a mucin-like sugar epitope modification of existing glycoproteins by a post-Golgi enzymatic activity that underlies myeloid cell maturation and differentiation[4].

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Two particularly important sugars found on the surface of myeloid cells are the CD15 antigen (or Gal-β(1-3)-[Fuc-α(1-3)]-GlcNAc; also known as Lewis x) and its sialylated analog, sialyl-CD15 (or sialic acid–α(2-3)-Gal-β(1-4)-[Fuc-α(1-3)]-GlcNAc; also known as CD15s or sialyl-Lewis x). These closely related structures have surprisingly different functions: neutrophils—a type of white blood cell in the myeloid lineage—use CD15s-selectin interactions to initiate cell adhesion with endothelial cells as part of the inflammatory response[5]. Once neutrophils have entered subendothelial tissues, the cell surface is altered to display CD15, which does not bind selectin, but instead serves as a ligand for the lectin DC-SIGN on dendritic cells. This pairing is one factor that neutrophils use to initiate dendritic cell maturation[6]; it engages both neutrophils and dendritic cells in attacking pathogens.

Though the functional significance of the display of either CD15s or CD15 is clear, the mechanism controlling which sugar is displayed was not known. The replacement of CD15s could result from vigorous new synthesis of CD15, thus diluting CD15s. Alternatively, CD15s could be directly digested by an endogenous sialidase and converted to CD15 through a new mechanism. Gadhoum and Sackstein now use inhibitors of specific points in carbohydrate synthesis to show that CD15 is surprisingly generated by the latter mechanism[4] (**Fig. 1**). Even more surprisingly, by measuring the sialidase activity of intact cells, the authors show that the majority, if not all, of the sialidase activity that cleaves CD15s is present on the cell surface.

To remove sialic acid from the cell surface, four distinct sialidases reported are considered[7]. Among them, only Neu-1 and Neu-3 can hydrolyze sialic acid conjugate. Gadhoum and Sackstein showed that Neu-1 is most likely responsible for the conversion of CD15s to CD15 by demonstrating that the levels of its transcripts and protein were increased

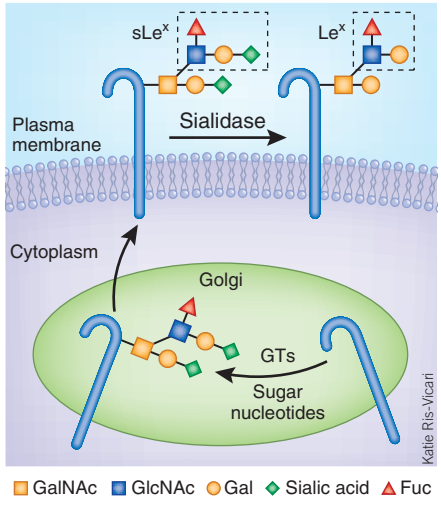

Figure 1 Biosynthetic pathway of mucin-like O-glycans. Once mucin-type O-glycans are synthesized in the Golgi by glycosyltransferases (GTs), the corresponding mucin-like glycoproteins are transported to the plasma membrane. Gadhoum and Sackstein showed that CD15s (sLe^x) on mucin-type glycoproteins is further subjected to processing by an endogenous sialidase on the cell surface, thus forming CD15 (Le^x). Proteins are shown in light blue.